turned-over ends of the lamellæ; through these liquid can readily be extracted when a freshly dissected cuttlebone is placed under reduced pressure. The yellowish membrane, which is closely applied to this region, not only has a copious blood supply but also contains numerous ampullæ close to the bone which are connected by small ducts to the veins. In the histological sections the ampullæ and ducts contain no blood although blood can be seen in blood vessels which are smaller than these. It therefore seems very likely that exchanges of liquid occur here in the living animal.

Cuttlefish which had been kept in the aquarium for a week or two exhibited the wide range of cuttlebone densities 0·48–0·71 (mean = 0·60, *S.D.* = 0·06, $N = 38$) whereas those freshly caught and studied about R.V. *Sarsia* had cuttlebones the densities of which fell within the much narrower range of 0·57–0·64 (mean = 0·62, *S.D.* = 0·02, $N = 17$). The cuttlebones differ, not in the weight of dry matter per unit volume which is always close to 38 per cent, but in the amount of liquid which they contain. Thus a cuttlebone of density 0·7 contains about 30 per cent liquid, whereas a cuttlebone of density 0·5 contains about 10 per cent liquid. The remainder of the cuttlebone contains gas; but this gas is always at less than one atmosphere pressure for when a fresh cuttlebone is punctured under sea water this enters very rapidly and the density of the cuttlebone increases. This uptake of sea water cannot come from the solution of a very soluble gas, for when a fresh cuttlebone is gently dried and punctured out of water, air enters very rapidly. Within the duration of our experiments, the mass of gas per unit volume of bone remained almost constant whatever the density of the cuttlebone, so that in a bone of reduced density the pressure of the gas is even lower than usual. There can be no question of an evolution of gas raising the pressure and expelling liquid. The gas within the cuttlebone therefore plays a completely passive part in the density changes.

The pressure of gas in the cuttlebone is in no way balanced against the external hydrostatic pressure as in the swim-bladder of a fish. The cuttlebone is not an impermeable structure and some other mechanism must therefore be present both to make the gas space initially, and to prevent liquid entering under the high hydrostatic pressures found where these animals live. We have taken liquid—this is principally a solution of sodium chloride—from the cuttlebone and found that it has a higher freezing point than either sea water or cuttlefish plasma (sea water and cephalpod plasma are very close to one another in total osmotic concentration, Robertson[2,3]). For example, in animals caught in about 70 m., where the maximum possible hydrostatic pressure pushing water into the cuttlebone would be about 7 atmospheres, the liquid extracted from the cuttlebone had about three-quarters of the concentration of salts found in sea water. This suggests that simple osmotic forces play a major part in keeping water out of the cuttlebone for between this liquid and cuttlefish blood there would be an osmotic effect balancing about 5 atmospheres of pressure.

On coming towards the surface the equilibrium between osmotic effect and hydrostatic pressure could be simply restored by the movement of a little water from the bone with the consequent concentration of the solution in the bone. This would make the bone a little less dense and the animal slightly more buoyant. This cannot be the whole story because in many animals kept in the aquarium the density of the bone remains high, whilst in others (in which the buoyancy mechanism is disturbed) the cuttlebones become far lighter (densities about 0·5) than this explanation would demand. In the latter the loss of liquid from the bone is greater than that needed simply to establish a new balance between osmotic effect and the reduced hydrostatic pressure. Moreover the liquid readily available for exchange seems to have completely gone from such a bone for it is impossible to extract any liquid even under very reduced pressure. Since water and salts have left the cuttlebone against both the hydrostatic gradient and the salt gradient there must exist a mechanism capable of pumping salts out of the bone.

Thus the cuttlefish possesses in the cuttlebone a buoyancy regulating mechanism of a very ingenious kind. It is true that is more bulky than the swim-bladder, it occupies about 9 per cent of the cuttlefish's volume whilst a swim-bladder occupies only 5 per cent of a marine fish; but it also serves as a skeleton. It is almost free from the main disadvantage of a swim-bladder in that its upthrust will change only slightly on quick change of depth. Its useful depth-range is probably limited, for even were the liquid within the cuttlebone to be pure water, the osmotic effect could only balance the hydrostatic pressure of about 240 m. of sea water. Although the osmotic effect could prevent water entering the gas spaces, the cuttlebone had nevertheless to withstand the full hydrostatic pressure of the water above the cuttlefish. It is very strong, and even when dissected from the animal and tested in a pressure tank it only implodes at pressures corresponding to depths appreciably greater than those at which the animals live.

We are grateful to Mr. Dobson of the National Institute of Oceanography who tested for us the behaviour of the cuttlebone under pressure.

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Age of *Carcharodon megalodon*?

Maurice Lariche[1] states that the teeth of the gigantic extinct shark, *Carcharodon megalodon*, found on the bottom of modern oceans belong to the Pleistocene period. The report of the Challenger Expedition[2] states that some shark teeth from the same station were covered with a layer of manganese dioxide—one slightly, another deeply. Among them at Station 281, depth 2,385 fathoms, South Pacific, were found teeth of *Carcharodon megalodon*. Knowing even approximately the thickness and rate of growth of the manganese dioxide layer, we should be able to establish the geological age of embedded teeth much more exactly than before. According to Hans Pettersson[3] the growth-rate is 0·15 mm.–1·4 mm. every 1,000 years and on the upper surface of manganese dioxide nodules it is about $1\frac{1}{2}$ times greater than on the lower.

The material for this investigation consisted of two teeth of *Carcharodon megalodon* from the above-mentioned Station 281. On the tooth *N*1 (Fig. 1) the manganese dioxide layer had almost completely disappeared, on the tooth *N*2 (Fig. 2) it covered quite a large area.

The thickness of the manganese dioxide covering was measured by a Swift petrological microscope. In the dental cavity, as usual for deep-sea deposits,

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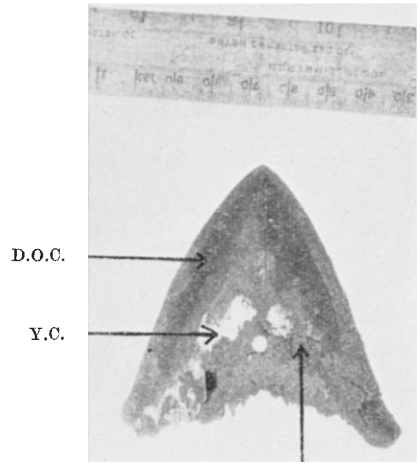

Fig. 1. Tooth *N*1, half natural size. D.O.C., deep olive colour; Y.C., yellow colour; 1700 μ, maximal thickness of manganese covering.

dentine disappeared and the manganese dioxide layer was much thicker than on the surface—up to 1 cm. But the results of Pettersson's work are scarcely applicable to this part of the manganese dioxide layer: he investigated the surfaces of nodules, and the rate of formation of the manganese dioxide layer inside the deposits cavity is a separate problem to be solved by special investigation. To determine the age of the teeth, we take the lowest rate of nodule formation and the greatest depth of covering only.

The colour of the main part of the front side of tooth *N*1 resembled the light-coloured yolk of a hen's egg varying from barite—yellow to buff—yellow, or apricot yellow and pale orange according to Ridgeway[4]. It is seen clearly on a photograph as a light field surrounded from the top and sides by a dark stripe and spots, deep olive in colour, which is characteristic of fossil shark teeth found in the ground and from the ocean bottom deeply embedded in manganese dioxide. Yellow seems to be a natural colour and indirectly shows that this tooth is geologically young. The manganese dioxide layer was preserved in several small spots only, the thickest of which is 1,700 μ. Using the minimal Pettersson's rate—0·15 mm. in 1,000 years—it will require 11,333 years for deposition.

Tooth *N*2 is much darker than the first one, the deep olive colour of the fossil predominating, the yellow (cream buff) colour occupying a noticeably smaller surface, which on the front side formed rather

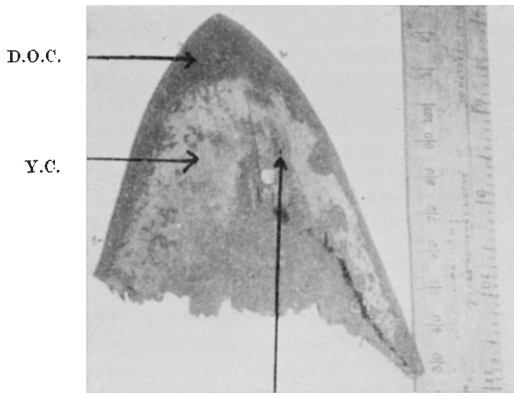

Fig. 2. Tooth *N*2, half natural size. D.O.C., deep olive colour; Y.C., yellow colour; 3640 μ, maximal thickness of manganese covering.

a small triangle in the middle. The maximal thickness of the manganese dioxide covering is 3,640 μ. The minimal rate gives an age of 24,206 years. This means that *Carcharodon megalodon* became extinct in the latest Pleistocene or even survived until the Holocene period. To prove this, let us imagine that *Carcharodon megalodon* was really extinct in the middle Pleistocene—about 500,000 years ago. Then even the minimal rate shows that its tooth must be covered with a manganese dioxide layer about 75 mm. in thickness. Or let us imagine that the tooth in 500,000 years was covered with a coating of 4 mm. only. Then the rate of manganese nodules growth will be literally microscopic, that is, 8 μ in 1,000 years. This small, quite speculative figure sharply contradicts all the results of Pettersson's experimental work.

Recognizing the relation between the age of the fossil and the thickness of the manganese dioxide covering, we can also determine the rate of nodule growth on the basis of the age of enclosed fossil. Up to 40,000 years, the carbon—14 method gives very exact results; but it needs a rather large quantity of organic material. This method is thence more suitable for such big deposits as whale earbones, covered with a manganese dioxide layer in the same manner as a shark tooth.

Knowing the age of the bone, we could estimate the overall rate of growth of the surroundings at different points and particularly in the deposit cavity.

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Distribution of Sodium in Compact Bone, as Revealed by Autoradiography of Neutron-Activated Sections

Recent work has emphasized the heterogeneous composition of compact bone. According to X-ray absorption, young osteons contain less calcium than the others[1]. Histological methods indicate that the mucopolysaccharides of the ground-substance are different in the preosseous layers, in the incompletely calcified osteons, and in the fully mineralized structures[2].

The distribution of sodium in compact bone was investigated in the same respect. Since histochemical methods are deficient in sodium, an attempt was made to identify this element after its activation in a nuclear reactor.

Pieces of ribs or tibiæ were taken from humans during surgical operations. They were kept in 96 per cent ethanol. It is obvious that part of the sodium, not firmly attached to the tissue, escaped in the fluid. Bones were cut in transverse sections with a saw, and these were ground down and polished by hand with emery paper.

The sections were submitted for six hours to a flux of $3{\cdot}5 \times 10^{11}$ neutrons/cm.^{2}/sec. in *Trico* (Triga Reactor supplied by General Atomic to the Government of Belgian Congo). It has been demonstrated[3]

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